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NUTRITION REVIEWS

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NUTRITION REVIEWS

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CULTURAL FACTORS IN NUTRITION

The study of cultural factors in nutrition has emerged only recently as a distinct focus of research, marked by the formation of the Committee on Food Habits of the National Research Council in 1941. The impact of culture on nutrition has been studied primarily in the realm of "food habits," *i.e.*, the second of two distinct spheres comprehended by the science of nutrition. The first includes biochemistry and physiology and is concerned with the determination of nutritional requirements and dietary standards. The other involves efforts by nutritionists and behavioral scientists to investigate actual food usages and to find effective means of adjusting habits to nutritional needs. Margaret Mead, Kurt Lewin and Natalie Joffe have published extensively on the latter sphere of inquiry.

The concept of *culture* as developed in anthropology refers to the learned or nonbiologic aspects of human existence, those transmitted through language and group life. In any given society, culture is the design for living developed by the group, a set of "regulations" governing the conduct of members. For the individual, culture acts as a screen of values and perceptions through which the person views food, his own body, and the world.

Cultural factors obviously and strikingly intrude in the service or "consumption" aspects of nutrition—diet therapy, nutrition education, and action programs on school, community, or larger scale. The "retailing" of nutrition usually involves efforts to influence food habits and ideas, sectors of culture which are often deeply rooted in custom, linked to emotional predispositions, and resistant to change. This point has been well documented and many of its implications explored in *Bulletins 108 (1943)* and *111 (1945)* of the Committee on Food Habits of the National Research Council.

The objectives of service nutrition may be frustrated unless cultural factors are taken fully into account. Nutrition education programs in schools may be cited as an example. They are often conducted in a well planned and skillful way within school settings. Nevertheless, they are sometimes carried out on the bases of implicit—and unwarranted—assumptions that cultural norms within pupils' families permit children to take roles as educators vis-a-vis their parents and to inspire the latter to alter the household's food pattern. Even should the assumptions hold for one group, there should be no expectation that they hold as well for others.

Beyond their relevance to service aspects of nutrition, cultural factors appear to impinge on at least three areas of nutrition research: (1) dietary surveys, (2) experimental nutrition with human subjects and (3) the epidemiology of malnutrition.

Dietary surveys. The essential point is that presurvey knowledge of and during-survey sensitivity to the social structure and culture of the group under study are essential to the successful handling of certain survey problems. In order to prepare survey forms for recording dietary intake and other data, workers require considerable prior knowledge of the group's foodways, food terminology, conceptions of what items do and do not constitute edible or reportable foods, and the kinds of questions that might give offense.

Should the survey sample be stratified or be assumed to come from a homogeneous population? Should survey workers be local or outside personnel; when and in what ways might each be effectively utilized? Both the sampling problem and that of personnel

utilization are manageable to the extent that the sociocultural profile is known and taken into account. Further, estimating the effects on survey results of having chosen a given number of days, particular days, or season; trying to select the "right" days or season in advance; and whether or when to make resurveys all involve knowing in detail, among other things, the community's cycles and time-patterns in diet. Finally, whether realistic assumptions can be made about given *individual* intakes on the basis of *family* intake data is a question to be decided not by logic or a standard conversion formula but by specific knowledge about prevailing patterns of intrafamily food distribution.

Experimental nutrition. Experimental work involving the cooperation of human subjects outside laboratory settings frequently faces cultural problems similar to those noted for dietary surveys. In addition, where analysis requires that samples of blood, excreta, or other body products be secured, or where it involves injecting substances into the body, special problems are sometimes encountered. In many societies subjects will tend to react with varying intensities of resistance to tamperings with the body and its products, reading their own cultural perceptions into objectively harmless procedures.

All peoples, whether or not formally schooled, have conceptions of the body and its workings which may or may not be in line with modern medical teaching. These concepts, or "body images," are not only part of the group's knowledge of man and nature but usually carry deep emotional and psychologic significance to individuals. Experimental workers in field settings should be forearmed with more than technical skill and efficiency—they should also be sensitive to the kinds of "body images" held by subjects and be prepared to adapt their technics and approaches accordingly.

Attention might also be drawn to a somewhat different relevance of culture to nutrition, *i.e.*, the possible utility of the storehouse of anthropologic literature for locating "natural experiments." Anthropology's concern with the firsthand study of culture, conducted in societies ranging in scale from primitive tribes to modern communities, includes an emphasis on providing a record of the total way of life, social organization and general adaptation to environment of the group studied. As a result, anthropologic literature comprises a fund of information on such topics as diet inventories, food production and preparation, dietary cycles and adjustments, activity patterns, general food habits and beliefs, health, and general utilization of the environment.

In short, the literature of anthropology might point to strategic groups or regions for testing a variety of hypotheses concerning dietary pattern and nutritional status. In this connection, it is of interest that certain current research on nutritional factors in atherosclerosis has an explicit cross-cultural design, in which a series of multiple variables are controlled by means of selecting subjects from cultures which are different in certain respects and similar in others. The cross-cultural "laboratory" permits less rigorous control than can be exercised with laboratory animals, but it does provide a variety of human populations whose arc of nutritional adaptations can be plotted against a great spread of dietary, environmental and other conditions.

The epidemiology of malnutrition. The role of culture in malnutrition can be illustrated by the example of kwashiorkor. Certainly, the fundamental problems to be solved in the attack on kwashiorkor have to do with clinical, pathologic, biochemical and dietetic features and relationships. In addition, cultural factors require investigation, since they appear to be implicated in causation, disease course and therapy.

Within its regions of occurrence in Africa, the incidence of kwashiorkor varies to some degree in accord with certain cultural variations: uses of the local plant and animal uni-

verse, dairy and agricultural traditions, spacing of pregnancies, distribution of available foods within the family, practices and schedules of weaning, and specific child-feeding patterns. Variations in these and other sectors of culture are presumably among the factors which affect the incidence and severity of kwashiorkor among groups with grossly similar backgrounds of race, economics and environment.

The course of the disease and its therapy are also modified by culture. What do the symptoms mean to people? Do they believe kwashiorkor *is* a disease? Do they recognize the entire syndrome as a disease entity, or do they fix attention on one or another symptom? What household treatment, if any, do they regard as appropriate? What are the prevailing attitudes toward medical and nutritional therapy? How and according to what cultural criteria do people respond to therapeutic and preventive diets? These and other questions are obviously of first order importance to any action program. The point is not to judge whether prevailing beliefs and practices are scientifically valid or bizarre; it is rather to determine in what ways they influence acceptance of action measures.

In summary, the service ends of nutrition, in that they attempt to influence food habits, are thoroughly immersed in culture. Dietary surveys are helped or hindered to the degree that a range of cultural phenomena within the groups studied is taken into account. Culture has at least two quite distinct bearings on experimental nutrition. For one, work with human subjects in the field may be affected by the operation of cultural factors. For the other, ethnographic accounts of a variety of cultures may identify "natural experiments" in nutrition. Culture also appears significant in the epidemiologic study of malnutrition, as exemplified by kwashiorkor.

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THE GROWTH OF CHILDREN ON VEGETABLE DIETS

In spite of great advances in nutrition during the past twenty or thirty years and much work done directly upon human subjects, our knowledge of the nutritional requirements of the human species is to a considerable extent inferred from experience with experimental and farm animals. Well designed large-scale feeding experiments with human subjects are so rare that they deserve the careful study of all, particularly when the results are as disturbing to modern teaching as the recent report by E. M. Widdowson and R. A. McCance entitled "Studies on the Nutritive Value of Bread and on the Effect of Variations in the Extraction Rate of Flour on the Growth of Undernourished Children" (*Med. Research Council Special Report Series No. 287, London (1954)*). As is well known, there was a major preoccupation at the beginning of World War II with the adequacy of the diets in the United States and Britain. The principal direct actions taken were the enrichment of bread in the United States and the elevation of the extraction rate to 85 per cent in Great Britain.

The growth studies reported in this publication were done in two orphanages in Duisburg and Vohwinkel in Germany in 1947 and 1948. A total of 169 children started in the experiments in Duisburg, and 141 in Vohwinkel. The main experiments lasted a year, and approximately 100 children completed the year at Duisberg, and 55 at Vohwinkel. The ages varied from 4 to 15 years in both institutions. The diets consisted of very small amounts of meat, fish, cheese and milk, such vegetables and fruits as were available on the German ration, and as much of the bread under test as the subjects desired. The total

animal protein intake was about 10 g. per day. Bread supplied 75 per cent of the calories, potatoes 6 per cent, soups and vegetables 15 per cent, and milk and other animal products, 4 per cent. In Duisburg five groups of children were used to study the following flours: 100, 85 and 70 per cent extraction, 70 per cent enriched to equal 100 per cent in thiamine, riboflavin, niacin and iron, and 70 per cent enriched to equal 85 per cent extraction. At Vohwinkel the three flours studied were 100, and 70 per cent extraction, and 70 per cent enriched to equal 100 per cent extraction. Various amounts of calcium were added to all of the flours so that the total calcium intake was unusually high. Twenty ml. of orange juice concentrate and vitamin A and D supplements were supplied to all children. Careful clinical, dental, and radiologic examinations were made at intervals. Heights and weights were determined at two-week intervals. Records of food intake were kept, and various laboratory tests were run including nitrogen, calcium, magnesium, thiamine, riboflavin, and niacin balance studies on selected subjects.

When the studies were started the children had been on restricted diets for some time, estimated to have supplied from 70 to 90 per cent of their calorie requirements. In general they appeared lively and energetic but small for their age. Many were thin and had pot-bellies. There was a considerable amount of skin sepsis, and hyperkeratosis of the upper arms was fairly common. Compared to the normal standard used (R. O'Brien, M. A. Girshick, and E. P. Hunt, *U. S. Dept. Agr. Misc. Publ. No. 366, Washington, D. C. (1941)*) the average height was about 95 per cent of normal, and the average weight was somewhat less, but varied with age. Twenty per cent were classified as having poor muscle development, and only 15 per cent as well-developed. All of the children were given an over-all clinical rating of either A, B, or C, with the scale established so that the majority were graded as B. Evaluation of the x-ray films of the hands showed a retardation in bone development which was 16 and 21 months behind the chronologic age in Duisberg and Vohwinkel respectively.

In drawing their conclusions the authors are extremely cautious, and state that the first conclusion to be drawn is that great caution must be exercised in coming to any conclusion at all. Nevertheless, these extensive studies led them to the conclusion that "no difference could be detected between the nutritive value of the different breads" in the studies upon the children. "Thus it has been shown that diets in which 75 per cent of the calories were derived from wheat flour and 21 per cent from vegetables, and which contained only 8 grams of animal protein per day, provided undernourished children aged 5–15 years with all of the nutrients required for a high rate of growth and development for a period of 18 months." The lack of differences between the various groups was striking. The children grew rapidly, particularly during the early months of the study, and at an over-all rate of about one and one-half times as fast as is expected for normal children in the United States. This rapid response is probably to be expected, since they were underweight at the beginning, but it supports the conclusion of the adequacy of the diet. The average calorie intakes were high during the first weeks, especially at Vohwinkel, and gradually decreased during the experiment. The over-all clinical ratings improved markedly and the scale, which was adjusted on the expectation that deterioration might occur, proved to be somewhat too high. Thus a number of children were graded A+ at the end of the study. (No child under 10 years reached this grade, however.) Similarly, the bone development, which was delayed at the beginning, was more rapid than normal during the experimental period. The diet thus appeared satisfactory in this respect also.

In view of the rather excellent growth and over-all performance, the biochemical studies are not of as great importance as might have

been the case had the children deteriorated. Nevertheless, some of the findings are of interest. As already indicated, the calcium intakes were so high that it is difficult to know what the findings mean in terms of requirements. Although the intake was less upon the 70 per cent extraction diets, the retention was considerably more than on the whole wheat or 85 per cent extraction diets. No differences in nitrogen retention were found. The urinary thiamine excretion more or less paralleled the intake. The lowest intakes were 1.27 and 1.07 mg. per day in the two 70 per cent extraction groups without enrichment, and urinary thiamine amounted to 13 and 18 per cent of the intake. Urinary excretion of riboflavin did not parallel intake and this suggests the occurrence of unavailable forms in whole wheat. Also, the excretion at Vohwinkel was extremely low, only 5 and 7 per cent at intakes of 1.02 and 1.34 mg. per day, while at Duisburg it was 18 per cent upon an intake of only 0.6 mg. per day. The data upon niacin derivatives in the urine also suggest unavailable or different forms in whole wheat and enriched flour. More N-methylnicotinamide appeared in the urine on the enriched diets, while the fecal niacin was much higher on the whole wheat diet.

At the end of the first year an additional experiment was organized using all of the children at Duisburg, some of whom had entered the orphanage since the main experiment was begun. The control group had a diet similar to that already described, and the bread was of 85 per cent extraction. This group obtained from 60 to 120 ml. of milk per day. The "milk" group was given 500 ml. of milk daily. The results of the clinical tests and changes in heights and weights after six months revealed no difference between the two groups. This study, coming at the end of the main experiment, further emphasizes the adequacy of the previous practically all-vegetable diet since no response to milk could be shown.

The diets used at Duisburg and Vohwinkel were also fed to rats and pigs to test the response of these species. When weanling rats weighing 40 g. were used, the results were approximately those that might have been expected from previous studies. The whole wheat diets from each institution promoted better growth than the corresponding white flour diets, but the white flour diet from Duisburg supported slightly better growth than either diet from Vohwinkel, including the diet containing whole wheat. When rats weighing approximately 150 g. were used, no significant difference in the different diets was demonstrable. Limited studies upon pigs which weighed 40 pounds at the beginning of the study showed no difference between the two Duisburg diets, but the pigs on the whole wheat Vohwinkel diet gained slightly more than those fed the white flour diet.

The original publication contains a great deal of information that cannot be mentioned in a review. However, the principal conclusion has been presented, namely that the basal diet composed largely of cereals appeared entirely adequate for rapid growth in children of these ages; no improvement from the inclusion of high extraction flours or enriched flours in place of white flour was evident; and the addition of milk to the diet also failed to improve the rate of gain or general performance. These data thus deserve serious consideration and strike at some of the fundamentals of modern nutrition teaching. It would have been helpful and informative if the data had been broken down into age groups. The data are generally presented as averages for the different dietary groups which contained children from 5 to 15 years of age, and it is not possible to compare actual intakes with recommended allowances or other standards. Also, it should be noted that the metabolic data were apparently all collected upon children 11 to 15 years of age. Since there is a suggestion, at least, in the report that the younger children showed somewhat less clinical improvement than the older ones, this may be of some importance.

It would appear that in no case were the nutrient intakes actually less than the minimum requirements wherever these are available for comparison. The findings thus do not seem to contradict the basic findings of nutrition. Modern nutritional standards have traditionally included as much as 100 per cent allowance for safety above the actual indicated needs. Since it can scarcely be doubted that the requirements for growing children are relatively high compared to those for adults, the necessity for such generous allowances is seriously questioned by this report. Similarly, the supposed inadequacies of vegetable protein diets for human beings, who grow much less rapidly than most experimental animals, are not supported by these data.

Obviously this report raises doubts as to the value of bread-enrichment programs. One important point should be borne in mind in applying the results to large population groups. In the institution the individual intakes were governed primarily by personal preference, not by the availability of food. On the other hand, in any large population economic, social and educational factors play important roles. The range of food intakes is thus much less in an institutional experiment than in the general population. D. M. Hegsted, M. F. Trulson, and F. J. Stare (*Physiol. Rev.* **34,** *221 (1954)*) have concluded that it is entirely reasonable for public health nutrition programs to be directed toward an improvement of a relatively small segment of the population consuming the worst diets. An evaluation of an "average" diet such as has been done by the Dutch workers J. F. Reith, A. Gorter, and M. Van Eekelen (*Voeding* **10,** *206, 244 (1949)*) and many others may not even be germane to the enrichment problem. The important factor is not the composition of the average diet, but the number of people who do not consume an adequate diet. Unfortunately, in dietary surveys to date no methods have been proposed which allow a valid estimate of this factor, since the low values encountered must include both error and true values. Also, as indicated by this report of Widdowson and McCance, what constitutes an adequate diet is still open to investigation.

The report should be greatly encouraging to much of the world concerned with serious nutrition problems. It is apparent that great improvements and perhaps adequate diets are possible under conditions which by most standards would appear inadequate.

STANNOUS AND FLUORIDE COMPOUNDS FOR TOPICAL DENTAL TREATMENTS

Various phases of the relationship of fluorides to tooth decay have been discussed previously (*Nutrition Reviews* **9,** *21, 80, 149, 228 (1951)*; **10,** *28, 74, 76, 114, 227 (1952)*; **11,** *167, 324, 353 (1953)*; **12,** *51, 77, 95, 156 (1954)*). Recently a series of reviews on different aspects on fluorides and tooth decay has been published in the form of a monograph ("Fluoridation as a Public Health Measure," J. H. Shaw, editor, *American Association for the Advancement of Science, Washington, D. C. (1954)*). From the data presented in various parts of this monograph, it is evident that the ingestion of proper amounts of fluorides in the drinking water during tooth development results in a 50 to 60 per cent reduction in the incidence of tooth decay, whereas the application of sodium fluoride solutions to the external surfaces of the teeth results in a 30 to 40 per cent reduction for a year or two after treatment.

Although water fluoridation is superior to topical fluoride treatments, almost half the population of the United States can never be reached by water fluoridation because they live in rural areas where fluoridation of the water supply is impractical. Hence there is

a continuing need to find some procedure that will be as effective as water fluoridation and that can be applied in areas where water fluoridation is impossible. Recent studies in rats to compare the effectiveness of sodium fluoride and stannous fluoride indicated that the latter was an appreciably more active agent in the prevention of tooth decay (*Nutrition Reviews* **12,** *51* (*1954*)) Therefore two studies have been conducted in which stannous fluoride and stannous chlorofluoride were used for the topical treatment of tooth surfaces.

In the first of these, C. L. Howell and J. C. Muhler (*Science* **120,** *316* (*1954*)) have described an experiment in which the topical application of a 1 per cent sodium fluoride solution was compared with the influence of the topical application of a 4 per cent stannous chlorofluoride solution. Approximately 800 children between the ages of 6 and 15 years served as the subjects. One half of the children were treated with a fresh unbuffered solution of sodium fluoride, while the other half received treatments with a fresh solution of stannous chlorofluoride. The procedure used was that described by J. W. Knutson (*J. Am. Dent. Assn.* **36,** *37* (*1948*)). The first fluoride application was preceded by a thorough prophylaxis. The remaining three treatments were given within the following ten days and were not preceded by prophylaxis. The treatment consisted in each case of keeping all surfaces of the teeth moist for a four-minute period by application of the appropriate solution with a cotton applicator. No control group was used in this experiment to determine the base line of caries index to be expected in this population of children.

It was noted upon re-examination after twelve months that the children whose teeth had been treated with the stannous chlorofluoride solution had approximately 85 per cent less newly decayed teeth and newly decayed tooth surfaces than the children in the sodium fluoride group. The children in the sodium fluoride group had an average of 0.71 newly decayed, missing, or filled surfaces, whereas the children whose teeth were treated with the stannous chlorofluoride solution had only 0.12 newly decayed, missing, or filled tooth surfaces. On the basis of numerous other studies on the topical application of sodium fluoride solutions, it was assumed that the sodium fluoride group had 40 per cent less tooth decay than comparable control children would have. This assumption would mean that untreated controls would have experienced 1.18 new decayed, missing, or filled surfaces in the year's period. Thus the effect in the stannous chlorofluoride group would represent a reduction of approximately 90 per cent from the hypothetic but probable value for the control group.

These data strongly suggest that the stannous chlorofluoride solution was a more effective agent for the topical treatment of tooth surfaces than sodium fluoride. Indeed, its effectiveness in this one-year period is comparable to the amount of reduction that is observed after the ingestion of fluoridated water during tooth development. However, because of the brevity of the observation period, it cannot be predicted from these data whether the influence may or may not be a more lasting one than the rather ephemeral effect of sodium fluoride treatments.

In a similar experiment, C. L. Howell, C. W. Gish, R. D. Smiley, and J. C. Muhler (*J. Am. Dent. Assn.* **50,** *14* (*1955*)) compared the effects of a 2 per cent stannous fluoride solution applied in two different ways with the influence of application of a 2 per cent solution of sodium fluoride. Twelve hundred children were represented in the total study, although only 620 subjects were used in the final tabulation of data. The final control group consisted of 139 subjects of elementary and junior high school age. Group A had 92 subjects whose teeth had been treated with a 2 per cent solution of sodium fluoride applied by the usual cotton applicator procedure. Group B consisted of 194 subjects to whose teeth the 2 per cent stannous fluoride

solution had been applied by the cotton applicator procedure. Group C consisted of 195 children whose teeth had been sprayed with the stannous fluoride solution. The preliminary examination for all four groups was accompanied by a thorough prophylaxis. The procedure for the three groups was repeated four times, with the applications from two to seven days apart. The final examinations were made two years after the topical applications were complete.

The group which received the sodium fluoride treatment had 36.3 per cent less new decayed, missing and filled tooth surfaces than the control group. The group which received the stannous fluoride application by the cotton applicator procedure had 58.8 per cent less carious lesions than the control group. Group C had 65.5 per cent less new decayed, missing and filled surfaces than the control group. Thus the stannous fluoride, independent of the procedure by which it was applied, was found to be significantly superior to sodium fluoride in reducing the dental caries experience of school children. It is noteworthy that the actual amount of fluoride that came in contact with the teeth in this study was 50 per cent less among the stannous fluoride groups than in the sodium fluoride groups. This is because a comparable weight of the total compounds was used rather than adjusted amounts to provide the same fluoride content in each solution. Obviously these data suggest, as in the first experiment, that the presence of the stannous ion had in some way augmented the action of the fluoride ion or had participated in the action itself. Again in this experiment the duration of effectiveness cannot be predicted.

The role of the stannous ion in these studies is unknown. Possibly it, like the fluoride ion, is adsorbed on the surface of the enamel in such a way as to alter the caries-susceptibility of the tooth surfaces. As yet the influence of the stannous ion during tooth development has not been reported. The earlier studies on the influence of stannous compounds on experimental dental caries were postdevelopmental in nature, so that the stannous ion could not have been incorporated into the tooth structure during calcification. Interestingly enough, the postdevelopmental studies in rats indicated that the stannous ion in the absence of the fluoride ion was ineffective as a caries-reducing agent. Whether or not this would be true with respect to topical applications to human teeth is unknown. This facet of the problem makes the interpretation all the more perplexing and interesting.

METABOLISM OF VITAMIN B_{12} IN PERNICIOUS ANEMIA

The concept of the etiology of pernicious anemia suggested by Castle postulated an extrinsic factor present in food which reacted with an intrinsic factor in the stomach to produce the erythrocyte maturation factor. Subsequent work has shown that vitamin B_{12} is both the extrinsic factor and the erythrocyte maturation factor and that the intrinsic factor is necessary for the absorption of vitamin B_{12}. Patients with pernicious anemia do not produce intrinsic factor and hence suffer from an impaired absorption of vitamin B_{12}. They may be treated with injected vitamin B_{12} or with the vitamin administered orally with intrinsic factor preparations (*see Nutrition Reviews* **12,** *4, 105 (1954)*).

Vitamin B_{12} occurs in serum in free form and also in combination with a plasma protein (*Nutrition Reviews* **12,** *164 (1954)*; **13,** *100 (1955)*). In addition, a considerable part of an administered dose is taken up by the tissues, particularly the liver (G. B. Jerzy Glass, L. J. Boyd, G. A. Gellin, and L. Stephanson, *Arch. Biochem. Biophys.* **51,** *251 (1954)*). In normal individuals only minute fractions of orally administered vita-

min B_{12} are excreted in the urine. Following injection of the vitamin the urinary excretion increases with increasing dosages and approaches 100 per cent when large doses are given. It seems probable that the urinary excretion is related to the concentration of free vitamin B_{12} in the serum and the low excretion following oral administration is due to slow absorption which does not exceed the binding capacity of the serum.

The relationship of serum vitamin B_{12} and urinary excretion of the vitamin to the hematopoietic response which follows its administration to pernicious anemia patients is largely unknown. Such information could conceivably shed some light on the nature of the metabolically active form of the vitamin.

G. B. Jerzy Glass, L. C. Lillick, and L. J. Boyd (*Bull. N. Y. Med. Coll.* **15,** *17 (1952)*; *Blood* **9,** *1127 (1954)*) have studied the relationship of serum and urinary vitamin B_{12} to the hematopoietic response. Patients with pernicious anemia in relapse and 2 patients with total gastrectomy were studied. Serum total vitamin B_{12} (bound plus free form) and urinary vitamin B_{12} were measured after the oral administration of the vitamin with and without intrinsic factor preparations, and after the intramuscular injections of the vitamin. Vitamin B_{12} was determined by microbiologic procedures after the samples were heated to convert the bound form of the vitamin into a form which would stimulate growth of the microorganisms.

A total of 7 patients with pernicious anemia in relapse were studied. After a preliminary observation period they were given from 10 to 1000 micrograms of vitamin B_{12} orally with and without an intrinsic factor preparation. The patients all exhibited the expected hematopoietic response to the oral vitamin B_{12} plus intrinsic factor. A sharp reticulocyte peak occurred followed by a gradual increase in red cell numbers. The orally administered vitamin B_{12} had little effect on serum levels of the vitamin, which were usually less than 0.2 millimicrograms per milliliter.

Following the oral administration of vitamin B_{12} with or without intrinsic factor, only extremely small amounts were excreted in the urine. The urinary excretion never exceeded 2 per cent of the administered dose. Most days there was no detectable amount of the vitamin in the urine. The only days a significant amount of vitamin B_{12} was excreted in the urine coincided with or immediately preceded the reticulocyte crisis. Since urinary vitamin B_{12} is probably related to the concentration of free vitamin B_{12} in the serum, this observation may indicate that the utilization of the vitamin in blood cell formation requires the conversion of the bound form into the free form.

In other experiments pernicious anemia patients were injected intramuscularly with from 30 to 100 micrograms of vitamin B_{12}. Following such injections blood levels rose rapidly and reached a peak of approximately 10 millimicrograms per milliliter two hours after injection. After eight hours the blood values had returned to the usual low values. In the first six hours following the injection approximately 60 per cent of the injected dose was excreted in the urine.

The patients with total gastrectomy responded to orally administered vitamin B_{12} in a fashion quite similar to the pernicious anemia patients with only slight changes in blood or urinary levels of the vitamin. Since these patients were not anemic no reticulocyte response was obtained.

Based on the minimum amount of injected vitamin B_{12} which will give a hematologic response in pernicious anemia patients, it can be assumed that the patients in these studies absorbed at least 0.5 micrograms of the orally administered vitamin per day. The urinary excretion amounted to only 10 per cent of this quantity. Hence the major portion of the absorbed dose was either stored in the body or else was metabolically so altered that it failed to stimulate growth of the assay organisms.

The results of these experiments reemphasize the striking difference in the

metabolism of injected and orally administered vitamin B_{12}. In the former case blood levels rise rapidly and a major portion of the dose is excreted in the urine, whereas in the latter situation blood levels rise only slightly if at all and only a fraction of the administered dose appears in the urine even in the presence of adequate intrinsic factor. The correlation between reticulocyte response and urinary vitamin B_{12} following oral administration of the vitamin may indicate that the free form of vitamin B_{12} is more closely related to the metabolically active product than is the protein-bound vitamin.

DIET, CHOLESTEROL AND ATHEROSCLEROSIS

Interest in the relation of dietary factors to blood cholesterol levels and to the pathogenesis of atherosclerosis continues. This is related in part to the increasing mean age of the population with an attendant increase in the medical importance of degenerative diseases, especially those of the cardiovascular system, and in part to technical advance which now allows for the application of new methods to the study of this problem. With regard to the latter, recent reviews have included discussions on the synthesis of cholesterol from acetate by mammals (*Nutrition Reviews* **7,** *233* (*1949*); **10,** *345* (*1952*)), the influence of fasting and of dietary factors on the rate of synthesis of cholesterol by liver slices *in vitro* (*Ibid.* **10,** *345* (*1952*)), the synthesis of cholesterol from orally administered C^{14}-labeled acetate in man (*Ibid.* **12,** *230* (*1954*)), and the application of the ultracentrifuge to a study of lipoprotein fractions in blood (*Ibid.* **8,** *176* (*1950*); **9,** *167* (*1951*)).

Several recent reviews have dealt with the relation of diet to blood cholesterol and atherosclerosis in man and in experimental situations (*Ibid.* **8,** *74* (*1950*); **10,** *168* (*1952*); **11,** *72*, *231* (*1953*); **12,** *25*, *220*, *270* (*1954*)), although the concept that atherosclerosis may be related to diet is not a new one (*Ibid.* **10,** *345* (*1952*)).

A report of observations on 5 apparently healthy male subjects ranging in age from 23 to 39 years has been made by G. A. Mayer, W. F. Connell, M. S. DeWolfe, and J. M. R. Beveridge (*Am. J. Clin. Nutrition* **2,** *316* (*1954*)). Evaluation of the institutional diets consumed by these subjects for some months prior to the experiment indicated daily intakes of cholesterol ranging from 300 to 1000 mg., fat from 80 to 150 g., calories from 2000 to 3000, and calories as fat from 33 to 50 per cent of total calories. The control mean plasma cholesterol level was 169 mg. per cent. After two weeks on a ration low in fat (11 to 19 per cent of calories) and in cholesterol (100 to 180 mg. per day) the mean plasma cholesterol level was 123 mg. per cent, and after four weeks it was 133 mg. per cent. During the fifth week eggs, calculated to provide approximately 800 mg. of cholesterol daily, were added to the diets and the proportions of fat were maintained. After one week the mean plasma cholesterol level was 129 mg. per cent. During the sixth week the initial experimental ration was supplemented with about 70 g. of vegetable fat as peanut oil, corn oil, or margarine, thus providing a low cholesterol, high vegetable-fat diet (with 34 to 46 per cent of the total calories supplied by fat). At the end of this study period the mean plasma cholesterol level was 150 mg. per cent. The diet employed for the last two weeks of the study was designed to provide a moderate cholesterol, high animal-fat intake, the latter being provided mainly as 70 g. of lard. Cholesterol intakes during this period ranged from 210 to 300 mg. daily. At the conclusion of this two-week period the mean plasma cholesterol level was 157 mg. per cent. In summary, the study was interpreted to indicate that a low fat, low cholesterol diet was accompanied by decreases in plasma cholesterol levels. The

addition of cholesterol in the form of egg yolk to the ration did not result in a change, but increases in dietary fat, whether of animal or of vegetable origin, led to increases in plasma cholesterol levels. The changes of serum cholesterol produced were small in reference to the variability of this quantity, so that the significance of these observations will be questioned.

During the course of nutritional studies of vegetarians, M. G. Hardinge and F. J. Stare (*J. Clin. Nutrition* **2,** *83 (1954)*) calculated the dietary intakes and determined the plasma levels of cholesterol in a group of lacto-ovo-vegetarians, "pure" vegetarians, and nonvegetarians. The dietary cholesterol was lower in the lacto-ovo-vegetarian than in the nonvegetarian group. Since "pure" vegetarians do not consume foods of animal origin their diets are free of cholesterol. The adult vegetarian groups had lower serum cholesterol levels than the nonvegetarian groups. The "pure" vegetarians displayed a significantly lower serum cholesterol level than the other groups despite a liberal intake of vegetable fat. A summary of data pertaining to the adult males included in this study will serve to illustrate these points. This study group included 15 lacto-ovo-vegetarians, 14 "pure" vegetarians, and 15 nonvegetarians. The mean daily caloric intakes were 3020, 3260, and 3720; of cholesterol 340, 0, and 914 mg. daily; and per cent of total calories from fat were approximately 33, 35, and 43, respectively. Plasma cholesterol levels in these groups were 243, 206, and 288 mg. per cent, respectively. The extent of differences in body fatness which is known to influence serum cholesterol level may complicate the conclusion that the dietary fat differences were responsible.

The South African Bantus comprise another population group which for economic reasons habitually consume rations of low fat content. Observations on the age trend of serum cholesterol concentrations in these groups in relation to fat intake were made by A. R. P. Walker and U. B. Arvidsson (*J. Clin. Invest.* **33,** *1358 (1954)*). It is estimated that the diets of these people provide less than 20 per cent of total calories as fat. Foods eaten are largely of vegetable origin. The fat intake of the Bantu averages about half of that usual in the United States.

Three groups of Bantus with low fat intakes, a group of Bantus consuming a partially Europeanized diet with higher fat content (20 to 25 per cent of total calories) and a group of European control subjects living in Africa with an estimated daily fat intake supplying 30 to 35 per cent of total calories were studied. The proportion of animal to vegetable fat in the latter two groups was not determined. The serum cholesterol was investigated in 218 Bantu subjects ranging in age from 15 to 93 years. Groups of rural Bantus subsisting on a low fat intake had low mean cholesterol levels. The Bantus consuming a Europeanized diet had a significantly higher mean cholesterol value than the rural Bantus. The cholesterol values for the European control group in the ages 21 to 50 years were comparable to those reported by A. Keys *et al.* (*J. Clin. Invest.* **29,** *1347 (1950)*) for an American population of similar ages, and were the highest of the five groups studied. For subjects over 40 years of age the Bantu demonstrated significantly lower serum cholesterol levels than the American population group. The authors argue that racial differences, state of health, caloric deficiency, low cholesterol intake, and pancreatic or liver dysfunction are not primarily related to the low cholesterol values observed in the African Bantu. It is concluded that the habitually low fat intake of the Bantu is responsible for the observed low serum cholesterol values in this population group, but the reservation is made that other unexplored factors in the *pattern* of diet may contribute to or be responsible for the observed differences. The specific suggestion is made that the high residue factor in the diet of the Bantu is deserving of further consideration. Here again it appears presumptuous to conclude that a difference of fat

intake is the critical factor, for the possible range of unmeasured influences which might act singly or might interact to influence cholesterol levels is very great.

Data concerning the incidence of atherosclerosis and coronary artery disease in the South African Bantu is presented in a companion publication (J. Higginson and W. J. Pepler, *J. Clin. Invest.* **33,** *1366 (1954)*). The records of 523 unselected necropsies at Baragwanath Hospital, which serves mainly the Bantus in the Johannesburg area, were reviewed. The findings in relation to the aortas and the coronary vessels were carefully analyzed. Patients with aneurysm of the aorta, syphilitic aortitis, or cancer were excluded. Patients with cancer, for reasons which are not evident, have a low incidence of atherosclerosis. Although it is impossible to apply sound statistical analysis to such population samples, the figures indicate that severe atherosclerosis of the aorta and coronary vessels is less frequent among the Bantu than among American or Danish hospital populations. This conclusion is in accord with the widespread clinical belief that complications of severe atherosclerosis (coronary and cerebral thrombosis, angina pectoris, intermittent claudication) are rare among indigenous Africans. Further support comes from the low frequency of deaths due to these complications among the Bantu compared to their frequency as reported for patients of similar age groups from American hospitals. It is suggested that the relative lack of atherosclerosis and its complications in the Bantu may be related to the low level of serum cholesterol found in these people who subsist on a low fat ration.

A relation between diet, serum cholesterol and atherosclerosis is well recognized. Available evidence points to the fat content of the diet as an important factor in this relationship. There is a paucity of information available concerning the relation of serum cholesterol levels and of atherosclerosis to other dietary factors such as total caloric intake, the bulk or residue of the diet, and relation of fat intake to that of other nutrients. Steps involved in the intermediary metabolism of cholesterol in man await more complete elucidation. Although a relation between dietary factors and atherosclerosis has been apparent for many years, the specific factors involved and their role in the genesis of atherosclerosis in man remain elusive.

NUTRITIONAL SURVEY IN RURAL JAMAICA

D. B. Jelliffe, L. L. Williams, and E. F. P. Jelliffe (*J. Trop. Med. Hyg.* **57,** *27 (1954)*) have described their findings from a clinical survey conducted in a rural village in Western Jamaica. The village contains something over 250 persons predominantly of African descent with varying degrees of Caucasian admixture, and is located in an area of poor soil and frequent droughts. Agriculture is the main occupation and the cash crops include citrus, coffee, coconuts and breadfruit. A few pigs and goats are raised along with very few cows. "Shop food," including flour, rice and corn meal, is used to a limited extent. The average weekly income is probably about $2.10 to $2.80.

The houses are constructed of red earth and lime. Roofs are of thatch, shingles, or zinc. The floors are of wood. Usually there are two rooms and as many as six people occupy a single room. Illegitimacy is common, but little or no stigma is attached to this and marriage is regarded as a symbol of a certain financial attainment. No schools are available in the village and the children irregularly attend others two or three miles distant. Approximately three quarters of the adult population are illiterate.

The water supply is inadequate and the villagers depend upon private tanks which frequently become exhausted. There are no springs or rivers and the village is too high

(400 feet altitude) for wells. During the dry season water must be carried two miles from the nearest storage tank. Recent construction of a number of latrines has greatly reduced the incidence of ancylostomiasis and other types of intestinal parasitism. A further check on helminthiasis is provided by the "deworming" of school children each term. Mosquito breeding and consequently malaria present little difficulty because of the lack of water.

The authors state that public health problems are not acute. Yaws occurs to a limited extent, but efficient treatment in recent years has caused a marked decline. The general health of the village appeared to be quite good and infant mortality is probably not high although exact figures are not available.

Quantitative dietary intakes were not determined but the general food practices are described. The infants are usually breast fed until about a year of age. The relatively long breast feeding is of great importance since, as in most tropical countries, alternatives to milk are difficult to obtain. D. B. Jelliffe (*Proceedings of the Joint CCTA/WHO Conference, Gambia (1952)*) has previously shown that the yield of breast milk falls rapidly after the sixth month in these poorly fed mothers. In any event even an abundant supply of breast milk fails to meet the needs of a rapidly growing child of this age and during the first year of life small quantities of starchy gruels and porridges are used. These may occasionally contain a small amount of sweetened condensed milk, the only kind which the poor of Jamaica can afford.

After weaning, the diet contains an excess of carbohydrate foods with only small quantities of condensed milk. A 10 ounce tin of this milk suffices for a week or ten days for the child of one to two years of age. Various kinds of solid foods are gradually added, such as banana, yam and breadfruit, although cornmeal remains the staple food. Various kinds of fruit juices are also widely used and carrot juice is extremely popular. Clinical carotenemia is occasionally seen in the outpatients. The adult diet consists largely of the starchy foods already mentioned, various tropical fruits, and some legumes. Foods of animal origin are seldom used owing to the expense. It would be of considerable interest to know the extent to which legumes are consumed since these would appear to be the nutritional salvation of the diet described.

The actual survey was made upon 252 persons estimated to be 87 per cent of the whole population. It consisted of height and weight measurements, approximate hemoglobin determination by the Tallqvist method, thick blood film for malarial parasites, direct microscopy of a single stool specimen for intestinal parasites, and a detailed physical examination. An extensive description of the nutritional appraisal, definition of terms, etc., is given. The authors emphasize the "doctrine of the common path." This is "the realization that many of the body tissues may only be able to react in a limited number of ways to widely differing stimuli, whether these be local trauma, exposure to ultra-violet light, biochemical lesions from vitamin deficiency, congenital abnormalities, etc."

The data are presented as the per cent incidence of the various lesions according to six age groups. The original paper will have to be consulted for the actual findings. The infants below 9 months of age showed little abnormality apart from a moderate anemia. Approximately a third of all the persons in all of the age groups showed a moderate degree of anemia. However, a great change was seen between the ages of 1 and 3 years. In a high proportion of the cases the previously well proportioned baby was replaced by a thin, puny and anemic infant with a palpable liver and definite dyspigmentation, silkiness, sparseness and straightness of the hair. Parasitic infestation appears to play little part in this picture and a high percentage of the group are believed to be passing through a postweaning phase of protein deficiency. This has been called latent kwashiorkor al-

though no case of manifest kwashiorkor was seen in the village at the time of the survey.

Among the older children the most striking features were the general thinness, lack of subcutaneous fat, and hypertrophied veins presumably resulting from carrying heavy loads of water. In spite of the thinness, the children were strong and wiry and mentally alert. Approximately a fifth showed hair changes. The usual anemia was found, and mosaic skin, permanent goose flesh and dyspigmentation of the face were common. The authors feel that these changes are directly related to the high carbohydrate diet, probably often deficient in calories, protein, certain B-vitamins, calcium and iron. A very high incidence of dental caries was found and it is suggested that this may be related to the low calcium intake, the consumption of highly milled carbohydrate and sugar, and the lack of dental hygiene. The latter factor was thought to be the probable cause of the relatively high incidence of gingivitis, since the intake of ascorbic acid was apparently adequate.

The general physique of the adults was considerably better than that of the children. Mosaic skin and permanent goose flesh were much less common and dyspigmentation and hair changes were not observed in adults. Gingivitis, caries, and edentulousness (toothlessness) were widespread. In the adult groups a progressive increase in the incidence of pterygia (thickened conjunctiva over part of the cornea), excess tissue of the bulbar conjunctiva, and cataracts occurred with age. The authors feel that these changes may be related to chronic exposure to sunlight although riboflavin or other nutritional deficiency cannot be ruled out. Thus the adult shows fewer stigmata of malnutrition than the child, presumably because his requirements are less and perhaps because of preferential treatment as far as food is concerned.

It is to be hoped that these extensive studies upon the physical status of a population that is clearly borderline with respect to some nutrients at some ages, at least, will be continued with careful records of dietary intakes. Field trials where specific nutrients are administered would be most informative. It is readily apparent that either dietary histories or physical findings are usually inadequate to determine the adequacy or inadequacy of the diet of a population group in terms of specific nutrients. The diet of the Jamaicans surveyed appears to be limiting in protein, yet the correlation between protein intake and the intake of other nutrients is often rather high and it cannot be considered as proven that the symptoms seen were actually due to protein deficiency. The actual amounts of various nutrients which must be provided to make the diet of the group studied satisfactory or reasonably satisfactory are unknown. Dietary histories in other areas have shown that many groups subsist reasonably well upon levels of intake much below those generally recommended. Populations such as those described by Jelliffe and his co-workers provide the opportunity for an estimation of the minimal nutritional needs in groups sufficiently large to be statistically valid. Although the difficulties of such an approach are clearly evident, nutritionists should soon take advantage of the opportunity.

COPPER, MOLYBDENUM AND SULFATE RELATIONSHIPS

A nutritional interrelationship between copper and molybdenum has been known for some time. Some recent reports from Australia indicate that the effects of molybdenum on copper metabolism are conditioned by the amount of sulfur in the diet.

A. T. Dick (*Australian Vet. J.* **28,** *30 (1952)*) found that liver storage of copper in

sheep was prevented by the inclusion, in an alfalfa hay diet, of only a few milligrams of molybdenum per sheep daily. The same amount of molybdenum, when added to a diet of oat hay, had no effect in limiting storage of copper in the liver. Dick further demonstrated that the alfalfa diet that permitted molybdenum to suppress copper storage in sheep liver was also responsible for a suppression of the molybdenum level in the circulating blood.

Subsequent studies by Dick (*Australian Vet. J.* **29,** *18 (1953)*) demonstrated that the factor in alfalfa which suppressed molybdenum in the circulation was inorganic sulfate. He further found that inorganic sulfate, together with molybdenum, was responsible for the prevention of copper storage in liver. This explained the apparent difference between the oat hay and the alfalfa hay diets, since the latter was found to supply about seven times as much inorganic sulfate as the former (Dick, *Ibid.* **29,** *233 (1953)*).

Three groups of 5 lambs each were fed an alfalfa hay diet and three comparable groups received the oat hay diet. One group on each diet received a copper supplement to bring the total daily copper intake up to 10 mg. per lamb daily. Another group on each diet received the same copper supplement plus 10 mg. of molybdenum per lamb daily. The third group on each diet received no supplement. The alfalfa hay diet contained copper and molybdenum to supply 7.0 and 0.5 mg. respectively. The diets were fed for three months. There were no significant changes in blood copper concentration, but marked changes in blood molybdenum concentration were noted. Pre-experimental levels of 0.5 to 3.0 micrograms per cent rose to 11.3 and 18.0 micrograms per cent respectively in lambs receiving the oat hay diet with and without added copper, and to 675.0 micrograms per cent on the same diet with added molybdenum. In contrast, the lambs receiving the alfalfa hay diet maintained normal blood molybdenum levels without added molybdenum, and the level rose to only 16.2 micrograms per cent in lambs on this diet with added molybdenum.

Changes in liver copper concentration were obtained from analysis of liver biopsy samples at the beginning of the experiment and after 114 days. Lambs on the alfalfa hay diet plus copper to supply 10 mg. of this element daily increased their liver copper store from an initial level of 373 to 540 parts per million (p.p.m.), or an increase of 167 p.p.m. (dry basis). In contrast, lambs receiving the oat hay diet and the same level of copper intake (10 mg. daily) increased their liver copper store from 361 to 857 p.p.m., an increase of 496 p.p.m.

Supplementing the diets with copper and with molybdenum (10 mg. each per lamb daily) resulted in a net decrease in liver copper store of 114 p.p.m. in lambs on the alfalfa diet, contrasted with a net gain in copper storage of 411 p.p.m. in the lambs on the oat hay diet.

Analysis indicated the alfalfa hay contained 0.3 per cent inorganic sulfate and the oat hay contained 0.05 per cent. In the next experiment the sulfate in the two diets was equalized by the addition of potassium sulfate to the oat hay diet. Twenty-four lambs were divided into eight groups of 3 each. Groups 1, 2, 3 and 4 received the alfalfa diet that supplied 2.2 g. of sulfate daily per lamb. Groups 5, 6, 7 and 8 received the oat hay diet supplemented to supply the same level of sulfate per lamb daily. All groups received a copper supplement to insure a 10 mg. daily intake while groups 1, 2, 5 and 6 received molybdenum to supply 10 mg. per lamb daily.

In this experiment positive liver copper storage was essentially the same on the alfalfa and oat hay diets without added molybdenum.

The molybdenum supplement under these conditions resulted in a net decrease in liver copper storage of 75 p.p.m. after eighty-two

days with both types of diets. Thus, the factor in alfalfa which, in combination with molybdenum, permitted a loss of liver copper could be imparted to an oat hay ration by increasing its inorganic sulfate content.

In further studies (A. T. Dick, *Australian Vet. J.* **30,** *196 (1954)*) the effects of sulfate and molybdate on blood copper levels in sheep were investigated more extensively. When the molybdenum intake was 15 mg. per sheep daily, sulfate intakes of 1.1, 1.8, 3.1 and 5.7 g. per sheep daily, respectively, produced increases in blood copper of 1.4, 1.1, 9.7 and 15.6 per cent, respectively. When the molybdenum intake was 90 mg. daily, the same sulfate intake levels produced respective blood copper increases of 25.3, 30.3, 46.1 and 74.1 per cent. Comparable results (intermediate in value) were observed when the molybdenum intake was 30 or 60 mg. per sheep daily.

These results established that for any given level of sulfate intake increases in molybdenum resulted in increases in blood copper level. Furthermore, at any given level of molybdenum intake, increases in sulfate intake resulted in increases in blood copper level.

The sulfate levels indicated were fed continuously for a two-month period prior to the molybdenum supplementation. The molybdenum was administered for three consecutive days. Blood molybdenum levels rose rapidly in all sheep during the three-day period when the above levels of molybdenum were administered. The blood molybdenum level was, however, affected by sulfate intake. For example, at the high-molybdenum intake levels, the sulfate intake level of 1.1 g. per sheep daily resulted in a blood molybdenum concentration of over 700 micrograms per cent, contrasted with a maximum concentration of only about 200 micrograms per cent when the sulfate intake was 5.7 g. per sheep daily.

The effect of sulfate and molybdate on wool fleece character was also investigated. On a diet supplying 80 mg. of molybdenum, 4.6 mg. of copper and 8 g. of sulfate daily, loss of crimp in the wool was observed within seven days, and a straight steely wool similar to that observed in copper-deficient sheep was produced during the six-week experimental period.

The development of a copper-deficiency lesion in the wool in the face of no apparent decline, and often a rise, in blood copper led Dick to explain these results as indicative that molybdenum limited the availability of blood copper for normal physiologic functions.

The results reported by Dick are of great interest and point again to the real importance of the dependence of one inorganic dietary constituent upon the relative concentration of others. The quantitative relationships between molybdenum and inorganic sulfate as they relate to copper deficiency remain to be established. Dick states he has early indications that the loss of function of pigmentation occurs at lower levels of molybdenum than does the loss of crimp in wool. The quantitative relationship between copper intake and the amounts of molybdenum and sulfate required to affect pigmentation is now under investigation by Dick.

It should be mentioned that the interrelationship of molybdenum and sulfate in copper metabolism points to the need for establishing sulfate concentration in animal feeds and pastures. This information is at present very limited.

FOOD YEAST PRODUCTION

An interesting and important trend to explore and develop nutrition resources for man and livestock has been evident in recent years. The food potential of various products of the sea was discussed recently (*Nutrition Reviews* **12,** *72 (1954)*). In a

variety of attempts to cope with present and increasing world feeding problems, investigators have studied the production of unicellular photosynthetic organisms such as *Chlorella*, the more intensive use of various parts of plant crops that are presently not efficiently used, and the potentialities of synthetic foods (*Ibid.* **12,** *111* (*1954*)).

The yeasts have received particular attention as supplements both to human foods and to animal (*Nutrition Reviews* **2,** *91* (*1944*); **7,** *86* (*1949*); **9,** *14* (*1951*)). In the early 1940's the Colonial Office of the British government formed a company to grow food yeast on a commercial scale in Jamaica as a means to create a new use for sugar cane and syrup (*Ibid.* **3,** *286* (*1945*)). The yeast in this case was *Torulopsis utilis*, which is preferred to baker's or brewer's yeast because of the greater economy of manufacture and the less readily detectable flavor of the product. The first plant of any size in the United States was started in 1948 at Rhinelander, Wisconsin. Recently it was announced that a second large new *Torula* yeast plant was soon to open in the United States, with the same species of yeast used in the Jamaica and Wisconsin plants (*J. Agr. Food Chem.* **3,** *17* (*1955*)). Both of the United States *Torula* yeast plants will use sulfite liquors from paper mills as the substrate rather than molasses. Sulfite liquor was the substrate used in German *Torula* yeast plants prior to and during World War II, when production reached 39,000 tons annually. This process is possible because the *Torula* yeast can utilize five-carbon sugars which comprise between 20 and 85 per cent of the sugar content of these waste products and which are unavailable to the *Saccharomyces* yeasts used in baking and brewing.

Each of the United States plants is capable of producing in the neighborhood of 11,000 pounds of yeast per day. At present the majority of the yeast produced in this fashion is used as a supplement in mixed feed for animals and poultry, but human food uses are being developed and account for about 20 per cent of the output of the plant now in operation. As yet *Torula* yeast does not appear on the grocers' shelves as such, but is combined in soups, sausage mixes, etc. It is interesting to note that both the *Torula* and *Saccharomyces* yeasts are roughly comparable in food value. Both contain about 50 per cent protein of good quality and are nutritionally quite acceptable in mixed diets. Diets containing yeast as a sole source of protein must be supplemented with methionine and with vitamin B_{12}.

It is interesting to note how much the gross composition of the yeasts can be varied by environmental circumstances during their production. Several examples of this are given by M. P. Steinberg and Z. J. Ordal (*J. Agr. Food Chem.* **2,** *873* (*1954*)), who studied the effect of fermentation variables on the rate of fat production by *Rhodotorula gracilis*. When a medium containing soluble nitrogen and a relatively high concentration of sugar is inoculated with the yeast, two distinct cultural phases can be observed. During the first, or "protein," phase the cells proliferate at a normal logarithmic rate and have a low fat content. In the second or fattening stage that begins with nitrogen depletion in the medium, the cell population remains nearly constant, but the fat content increases. In the inoculum that was placed in a fermentor at pH 4.5, the quantity of dry yeast amounted to 0.026 mg. per 10 ml. Only 5 per cent of this was fat. The relation of fat to nonfat dry yeast was also used as an expression of the situation that existed. At the beginning of fermentation the fat ratio was 0.053. At the end of the thirty-eight-hour fermentation the fat ratio had increased to 0.825.

It was found that pH had a particularly striking effect upon the rate of fat production when the range from 3.0 to 8.5 was tested. At a pH of 3.0, the rate of fat production was linear from the beginning until the termination. However, at a higher pH, particularly at 8.5, there was a prolonged lag up until about eleven or twelve

hours, during which there was little fat production. Thereafter fat production was much more rapid than at the lower pH until the peak was reached about thirty-two hours after inoculation. The effect of temperature was tested at 22°, 28° and 35° C., at a pH of 7.5. The maximum rate of fat ynthesis occurred at 28° C., the optimum growth temperature for this organism. Reduction of the temperature to 22° C. reduced the fat production rate to less than one-half that obtained at 28° C. When the yeast was grown in a substrate from which the yeast extract usually present as a source of growth factors had been omitted, there was limited growth of the yeast organisms, but the normal fattening process occurred. This indicates that, if additional accessory factors are necessary for fat synthesis beyond what are contained in the cell, the requirements are very low.

Experiments in which the sugar concentration of the mediums was varied between 4 and 40 per cent by weight indicated that all concentrations up to approximately 20 per cent were equally effective in promoting fat synthesis. Above this concentration, the fat production decreased precipitously. Glucose, fructose, and an equal mixture of glucose and fructose were tested as substrates for fat synthesis and found to result in nearly equal amounts of fat production. These data suggest that carbohydrates such as starch, sucrose, or inulin, after proper hydrolysis, would be of equal value as substrates for fat synthesis.

Ethyl alcohol and sodium acetate in concentration equimolar with the sugars in regard to carbon atoms were used as substrates. Although in both cases an appreciable amount of fat was formed, the ultimate fat contents were much lower than when a sugar was used as the substrate. In a second experiment glycerol and sodium acetate were used as adjuncts to corn sugar, replacing 10 per cent of the normal sugar concentration on a carbon basis. Samples containing added glycerol showed dry cell and fat contents after twenty-nine hours almost identical to the corn sugar control. The yeast suspensions containing acetate were less satisfactory than the sugar control. Sodium, calcium and ferric ions did not need to be added to the medium as sufficient appeared to be present in the crude sources of carbohydrates commonly used in the substrate.

These studies indicate that the composition of yeast can be controlled rather closely depending upon whether the current need is for fat or for protein production. They also suggest the wide variability possible in the substrate to be used by the organism. Presumably shifts could frequently be made in the substrate to make use of what is currently most available and economical.

METABOLISM OF THIAMINE

Nutrients that are required only in catalytic quantities have biochemical and physiologic activities that are often relatively easy to assess. The metabolic breakdown of the nutrient itself, however, may present a far more difficult problem. Determination of blood levels and excretion patterns following a test dose under standardized conditions has yielded some insight into the course such nutrients follow in the organism. Interpretation of such data is always complicated by body stores and possible synthesis in the animal. The use of compounds isotopically labeled in key positions affords a greatly improved means of following metabolic breakdown and turnover in the intact animal.

P. T. McCarthy, L. R. Cerecedo, and E. V. Brown (*J. Biol. Chem.* **209,** *611 (1954)*) investigated the fate of thiamine labeled with S^{35} in the rat. In the first experiment young male rats weighing approximately 225 to 275 g. were maintained on a synthetic diet containing 20 mg. of thiamine hydro-

chloride per kilogram for five weeks. A single dose of 50 micrograms of radioactive thiamine was injected intramuscularly. Thiamine was then omitted from the diet for the following ten days, during which time urine was collected to determine the distribution of the radioactive sulfur in the urine. Values for inorganic sulfate, ethereal sulfate, and neutral sulfur are reported.

Radioactivity amounting to 2.33 per cent of the original dose of thiamine was excreted as inorganic sulfate during the ten-day period, indicating thiamine breakdown followed by complete oxidation of the sulfur in the thiazole moiety of the molecule. Only very small amounts of radioactivity were found in the ethereal sulfates. The neutral sulfur fraction of the urine represented 62 per cent of the injected dose. Thiamine itself accounted for about 60 per cent of the neutral sulfur fraction. The remainder, which was not further characterized, represented molecules in various stages of breakdown between thiamine and inorganic sulfate. These compounds did not form thiochrome under the conditions for thiamine assay.

A total of 64 per cent of the dose was recovered from the urine during the ten days of collection. S^{35} excretion was detected in the urine within the first twenty-four hours after administration of the injected dose. The concentration of radioactivity was higher in each of the sulfur fractions during the first half of the collection period than during the second half. During the first five days of collection the ratio of radioactive to nonlabeled thiamine was 1:31, whereas in the second five days the dilution ratio was 1:70, indicating increasing depletion of the radioactive thiamine.

The second experiment was carried out in a similar manner except that the animals were maintained on a thiamine-free diet for three weeks, at which time signs of thiamine deficiency were evident. One group of rats received 20 micrograms of radioactive thiamine by intramuscular injection and the second group received a 50 microgram dose. The latter group was killed after five days and analyses for S^{35} were carried out on several organs and tissues. The rats that received the 20 microgram dose excreted approximately twice as much S^{35} in organic sulfate as the thiamine-sufficient rats in the first experiment, although the absolute quantity was still small. Conversely, smaller quantities of S^{35} were excreted as neutral sulfur in each of the two five-day collection periods (a total of 46 per cent). Very small amounts of radioactivity were found in the feces. This measurement was not made in the first experiment. A total of 53 per cent of the total injected dose was recovered after ten days. Of the thiamine-depleted animals that received 50 micrograms of thiamine-S^{35} by injection, the total radioactivity recovered from the excreta after five days was 52 per cent.

The requirement of rats for thiamine has been placed at 200 micrograms of thiamine per kilogram of diet. On this basis one would expect rats the size of those used in these experiments to consume approximately 40 micrograms of thiamine per day. The doses of radioactive thiamine that were used in these experiments therefore represent reasonable quantities for daily intake. These results can be considered indicative of normal breakdown of thiamine, whereas very high test doses might result in altered excretion patterns. The organs and tissues contained amounts of S^{35} in the following decreasing order: muscle, testes, kidneys, liver, heart, brain, lungs, thymus, spleen, and seminal vesicles. In general, highest concentrations of S^{35} were associated with known storage sites of thiamine.

Injection of the thiamine antagonist oxythiamine twenty-four hours after injection of 20 micrograms of labeled thiamine in depleted rats caused a higher percentage of S^{35} to be excreted in the neutral sulfur and a smaller quantity in the total sulfate fraction. These results indicate a similarity of response by the rat under conditions of thiamine deficiency as compared with the effect of the thiamine antagonist. On the

other hand, the animals receiving adequate thiamine degraded this vitamin somewhat differently quantitatively.

H. Borsook *et al.* (*Proc. Nat. Acad. Sci.* **26,** *412* (*1940*)) injected thiamine-S^{35} into a normal man after a period on an adequate diet and again after thirty-six days on a thiamine-free diet. The excretion of radioactive sulfur in urinary neutral and inorganic sulfur as well as S^{35} in the feces was determined. While on a normal diet, the subject was injected with 16 mg. of thiamine-S^{35} per day for four days. This quantity of thiamine is about sixteen times higher than the daily requirement. After six days 61 per cent of the S^{35} was recovered in the urine and 11 per cent in the feces. From the thirty-seventh to the forty-sixth day on the thiamine-free diet the subject was injected with 2.7 mg. of thiamine-S^{35} per day. No S^{35} was detected in the urine during the first two days after the first injection. Nonradioactive thiamine excretion via the urine increased immediately even though none had been excreted during the previous thirty days. It was concluded that in thiamine deficiency appreciable quantities of the vitamin are retained in the tissues. Only 26 per cent of the injected S^{35} was recovered in the urine under these conditions of deficiency as compared with 61 per cent in the complete diet experiment. While the subject was on the deficient diet, half of the nonthiamine radioactive sulfur excreted in the urine was in the neutral and half in the inorganic sulfur. On the normal diet the urinary excretion of S^{35} in the inorganic sulfur was approximately one-third higher than the neutral sulfur.

The results of these two experiments were similar under conditions of adequate thiamine intake; however, a higher proportion of the injected thiamine was retained under conditions of thiamine deficiency in man than in the rat. Differences in the relative size and number of injections as well as species may account for these dissimilar results. In men maintained on a constant thiamine intake for periods of two to eight months, O. Mickelsen, W. O. Caster, and A. Keys (*J. Biol. Chem.* **168,** *415* (*1947*); *Nutrition Reviews* **5,** *296* (*1947*)) observed wide variations in excretion patterns between individuals, particularly in the range of adequate intake. They recovered 30 to 40 per cent of the dietary thiamine in the urine (as thiamine and pyramine, the pyrimidine moiety of the thiamine molecule) at intakes of 0.61 to 1.00 mg. of thiamine per day. As the intake increased, the per cent recovery gradually decreased.

From these studies on urinary excretion of S^{35} from labeled thiamine, it has been shown that part of the thiamine in the body is broken down extensively. Small but significant amounts of sulfur were completely oxidized to inorganic sulfate, whereas negligible quantities were found in ethereal sulfates. Partial breakdown products, which were not further characterized, as well as thiamine itself accounted for the major pathway of excretion.

These studies add significantly to the knowledge of thiamine metabolism. In view of the wide variability which has been observed between individuals in their excretion patterns, it appears wise to accept these findings with reservations until more are available. Through the use of radioactively labeled thiamine coupled with pyramine determinations it will be possible to amplify these studies, particularly the specific effect of other nutrients upon the manner in which thiamine is metabolized.

ELECTROLYTE DISTURBANCES IN POTASSIUM DEFICIENCY

A potassium deficiency in rats produces a decrease in potassium and an increase in the sodium concentration of skeletal muscle and erythrocytes. The chloride level in the

plasma is reduced, with a concomitant increase in bicarbonate producing a state of alkalosis.

During the past few years a number of studies have attempted to elucidate the metabolic changes that occur in animals fed a potassium-deficient diet. R. Schwartz, J. Cohen, and W. M. Wallace (*Am. J. Physiol.* **172,** *1 (1953)*) fed weanling rats a synthetic diet containing less than 1 millimole of potassium per kilogram of diet. The animals receiving this diet supplemented with potassium gained weight at the rate of 35 g. per week. The animals on the deficient diet gained for a few days and then declined to their original body weight. At the end of fourteen days deficient animals showed shaggy fur, abdominal distention and diarrhea. On autopsy, the gastrointestinal tract was dilated and gross cardiac lesions were evident. The level of potassium in the serum was reduced from 4.2 millimoles per liter to 1.5; chloride concentration was reduced from 110 to 95 millimoles per liter; the sodium level, however, increased from 148 to 156 millimoles per liter.

Calculations by the above workers indicated that the muscle probably accounted for most if not all of the potassium lost from the body. Although there was a marked reduction in the concentration of the potassium in the plasma, this was partially compensated for by an increase in the total extracellular fluid volume.

Studies similar to the above have been carried out by E. Muntwyler, G. E. Griffin, and R. L. Arends (*Am. J. Physiol.* **174,** *283 (1953)*). These workers were impressed by the interdependence of potassium and protein nutrition reported by P. R. Cannon, L. E. Frazier, and R. H. Hughes (*Metabolism* **1,** *49 (1952)*; **2,** *297 (1953)*; *Nutrition Reviews* **12,** *75 (1954)*). For this reason they investigated the influence of potassium deficiency (0.04 millimoles of potassium per 100 g. of diet) upon the nitrogen balance of adult male rats. The animals receiving adequate potassium retained 108 mg. of nitrogen per day; on the potassium-deficient diet the rats stored only 50 mg. of nitrogen per day. This observation supports the thesis that in the absence of adequate dietary potassium, nitrogen is wasted.

An inverse relation between nitrogen balance and dietary chloride was seen on the low potassium diet. The rats on the diets containing a low level of chloride stored three times as much nitrogen as those on the high chloride diet.

Future studies should include carcass analyses in order to determine the changes that occurred in the animals which stored nitrogen on the low potassium diet in spite of a loss in body weight. The rats on the low potassium diet containing a high level of chloride lost 26 g. in thirty-five days while retaining nitrogen equivalent to approximately 35 g. of body tissue.

In order to determine what role the kidneys played in the loss of body chlorides during a low potassium feeding regimen, D. C. Darrow, R. E. Cooke, and F. E. Coville (*Am. J. Physiol.* **172,** *55 (1953)*) analyzed the kidneys of their potassium-deficient rats. There were no changes in the concentration of potassium, sodium, chloride, phosphorus, nitrogen and water in the kidneys. It is unfortunate that the diet used by these workers contained ten times as much potassium as that used by Schwartz and coworkers (*loc. cit.*).

The work on the kidneys was extended by A. Brokaw (*Am. J. Physiol.* **172,** *333 (1953)*). She observed the typical signs of potassium deficiency in weanling rats two weeks after starting the deficient diet. The potassium concentration in the muscles of her deficient rats was reduced from a control value of 60.5 millimoles per 100 g. of fat-free solids to 32.6 millimoles. (It should be noted that Brokaw's value for the normal potassium level in rat muscle is much higher than that reported by Schwartz *et al.* (*loc. cit.*) which was 49.0, and by Darrow *et al.* (*loc. cit.*) which was 48.9. Brokaw's value for the muscle from the deficient rats is

similar to that reported by Schwartz *et al.*, 31.4 millimoles, but lower than that of Darrow *et al.*, 38.4 millimoles).

Brokaw secured only a few indications of kidney disturbance in her potassium-deficient rats. The relative size of the kidneys was increased from a control value of 1.2 per cent of the body weight to 2.0 per cent. Associated therewith was a slight disturbance in ability to excrete water. This, however, was not apparent when only moderate amounts of water were given. Under such circumstances both the potassium-deficient rats and the pair-fed controls excreted the same amount of urine. This was also the case when moderate amounts of water were given together with pituitrin (to promote diuresis). Not only were the kidneys able to excrete moderate amounts of water, but they were also able to concentrate urine in a normal fashion when water was withheld.

Alterations in kidney function were not seen until excessive doses of water were given. Two of the 5 deficient rats died before the full dose of water had been administered. One of the survivors showed evidence of water intoxication (slight convulsions). All of the control rats survived; none of them showed the discomfort seen in the deficient animals. Three hours after the first dose of water, the surviving potassium-deficient animals had excreted 17 to 19 per cent of the dose, as compared to 40 to 44 per cent excreted by the pair-fed controls.

Recently M. Iacobellis, E. Muntwyler, and G. E. Griffin (*Am. J. Physiol.* **178,** *477 (1954)*) extended the studies on potassium deficiency to include changes in kidney enzymes. These workers used the following diets: (1) low in potassium, (2) low in protein, and (3) low in both potassium and protein. These diets were fed to rats weighing between 250 and 300 g. for a period of five weeks. At the end of this time, the rats on the low potassium diet showed only a slight loss of body weight, whereas the rats on the other two diets lost approximately one third of their original weight. The relative size of the kidneys was increased only on the diet that was low in potassium. Where the diet was low in both potassium and protein or only in protein, the size of the kidneys was normal. The concentration of nitrogen when expressed on the basis of the fresh weight of the kidneys was the same for two groups; the one on the low protein diet showed a slight reduction (27.6 versus 31 mg. of nitrogen per gram of kidney).

The studies on enzyme concentration showed that the glutaminase and carbonic anhydrase activities paralleled each other but did not follow the changes in total kidney nitrogen. The concentration of glutaminase in the kidneys was increased from a control value of 2900 to 8000 units per gram of nitrogen for the potassium-deficient animals; similarly, the carbonic anhydrase activity was increased from 26 to 68 units. Supplementing the diets of the deficient rats with potassium for seven days restored the enzyme levels practically to normal. The animals that were on the low protein and low protein-low potassium diets had the same concentration of these enzymes in their kidneys as the control rats.

The increased ammonia excretion of the potassium-deficient rats (1.85 mEq per twenty-four hours versus 0.89 mEq per day for the controls) might be explained on the basis of the increased glutaminase concentration in the kidneys. The changes in the glutaminase concentration and ammonia excretion in the potassium-deficient rats appear paradoxic since these rats are in a state of alkalosis as shown by the increase in their plasma bicarbonate levels (35 mEq per liter versus a normal level of 25 mEq per liter). Such a state of alkalosis should be associated with a reduction of kidney glutaminase concentration and ammonia excretion (B. M. A. Davies and J. Yudkin, *Biochem. J.* **52,** *407 (1952)*).

The concentrations of D-amino acid oxidase and arginase in the kidneys of the

potassium-deficient rats were normal when expressed on the basis of nitrogen content. This was also true for the rats on the low protein and low protein-low potassium diets.

The above studies aid in suggesting some of the metabolic defects existing in a potassium deficiency, but the results indicate that more work will have to be done before the defects can be pin-pointed. Before definitive answers can be secured to any phase of this problem, it will be essential to determine whether some of the electrolyte disturbances reported for a potassium deficiency are real, or analytic aberrations. The need for such a study is emphasized by the differences reported by three of the above groups of workers for the potassium concentration in muscle in control and deficient rats. Similarly, it may be necessary to see if such factors as dietary alterations, severity of the potassium deficiency, the genetic background and the age of the animals have any influence on the results. It also would appear desirable to study the changes in body composition that occur in a potassium deficiency. Since Muntwyler and co-workers (*loc. cit.*) noted positive nitrogen balances in their potassium-deficient rats while they were losing weight, there must have been profound changes in body water or body fat, or in both.

Potassium deficiency in rats produces a marked loss in muscle potassium concentration. This is the primary source of the potassium that is lost from the body in that deficiency, although there is also a reduction in the concentration of potassium in the plasma.

There are disturbances in kidney function in potassium-deficient rats as shown by the reduction in plasma chlorides and the increase in bicarbonate. The above studies suggest that the alterations in the kidney are of a subtle nature as indicated by the fact that it was only when large amounts of water were given that a difference between the deficient and control animals appeared.

IODINE AND THIOURACIL ACTIVITY

Thiouracil is one of a number of compounds which, when fed to an animal, interfere with the production of thyroxine and cause enlargement of the thyroid gland. Dietary iodine opposes these effects of thiouracil. A detailed study of this relationship has been made by D. R. Highley, H. E. Parker, and F. N. Andrews (*J. Nutrition* **54,** *249 (1954)*).

In one experiment weanling male rats were fed diets containing iodine at levels of 20, 100, 400 and 1600 parts per billion of diet. The feeding period was of six weeks duration. During the last two weeks of the feeding experiment the diets were supplemented with none, 0.005 per cent, 0.025 per cent, or 0.1 per cent thiouracil. By the use of sixteen groups of animals the effect of each level of thiouracil could be studied at each of the four iodine levels. The basal diet consisted of ground yellow corn 58.6 per cent, wheat germ 35.0 per cent, salts 4.0 per cent, cottonseed oil 2 per cent, methionine 0.4 per cent, along with a supplement of vitamins. The basal ration was analyzed for iodine and found to contain 20 parts per billion. Higher dietary levels were obtained by adding potassium iodide to the ration.

When the diet contained iodine at a level of 20 micrograms per kilogram of diet (20 parts per billion), the addition of a small amount of thiouracil (0.005 per cent) caused an increase in thyroid weight but did not affect growth, thyroid iodine content, or adrenal weight. The higher levels of thiouracil depressed growth and decreased the iodine content of the thyroid while increasing the size of the gland.

High levels of dietary iodine rendered the thiouracil less effective. However, even 1600

micrograms of iodine per kilogram of diet did not suffice to counteract completely the growth-inhibiting effect of 0.1 per cent thiouracil. Nor could it prevent entirely the thyroid enlargement caused by this high dose of thiouracil.

The second experiment dealt somewhat with the time relationship of iodine and thiouracil administration. When weanling rats were fed the iodine-deficient diet (20 micrograms per kilogram) for four weeks prior to thiouracil administration, weight gain was adversely affected in spite of a simultaneous twenty-fold increase in the amount of dietary iodine. Marked thyroid enlargement was also seen in these animals. Feeding liberal amounts of iodine (400 micrograms per kilogram) for four weeks prior to thiouracil administration largely prevented the adverse effects of the goitrogen no matter what level of iodine was fed during thiouracil administration.

Microscopic examination of the thyroid glands showed that even the lowest level of thiouracil (0.005 per cent) caused hypertrophy and hyperplasia regardless of the level of iodine administered simultaneously. The minimum iodine requirement of these particular rats under the conditions employed by the authors was estimated to lie between 100 and 400 micrograms per kilogram of diet. In this range of iodine intake the enlargement of the thyroids appeared to be proportional to the log log of the thiouracil dose, as previously reported by D. Bargeton, C. Krumm-Heller, and F. de Fombelle (*Compt. rend. Soc. de biol.* **144,** *650 (1950)*).

PROTEIN DEFICIENCY AND PREGNANCY IN THE RAT

The importance of an adequate protein intake in maintaining normal pregnancy is widely recognized. The pathogenesis of the harmful effects of protein deficiency in pregnancy are as yet incompletely understood. The experimental studies of M. M. Nelson and H. M. Evans (*J. Nutrition* **51,** *71 (1953)*) bear on this problem. Female rats 3 months of age were placed on purified diets on the day of breeding. Diets were prepared containing from 0 to 25 per cent of protein provided as alcohol-extracted casein. Supplements of crystalline vitamins in adequate amounts were incorporated into the diet. In some experiments the vitamin content of the diet was doubled to rule out the possibility of developing vitamin deficiencies due to reduced food intake.

The course of pregnancy was normal in female rats ingesting diets containing 25, 20, 15 and 10 per cent protein. There was, however, a progressive decrease in the maternal weight gain during pregnancy, from 120 g. for females eating diets with 25 per cent protein to 82 g. for rats eating the diets containing 10 per cent protein. When the protein content of the diet was further reduced to 5 per cent, resorption of the implantation site occurred at 30 per cent of the sites and 17 per cent of the young were born dead. On this diet the female rats gained only 11 g. Doubling the allowance of crystalline vitamins improved the ability of these rats to bear young. The resorption of implantation sites increased greatly when the dietary protein was reduced to 2.5 per cent and finally when the diets were protein-free. A weight loss of major proportions occurred under these conditions of protein deficiency. Macroscopic congenital abnormalities were not found in the young of females given low protein diets. The average weight of the young rats decreased from 6.5 g. with mothers ingesting a diet containing 25 per cent protein to 2.9 g. for the young from mothers given the protein-free diet.

The possibility that the observed results could be explained simply on the basis of inanition was examined. Pregnant rats were

fed the same weight of the high protein diet as was eaten by the rats receiving the low protein diet. The reproductive performance of the pair-fed rats given 30 per cent dietary protein was normal. When the pair-fed rats were given a 20 per cent protein diet the results were nearly as good, only 11 per cent of the implantation sites being resorbed.

The possibility that the failure to produce the necessary hormones of pregnancy was the cause of the fetal deaths during protein deficiency has been considered in a subsequent publication (M. M. Nelson and H. M. Evans, *Endocrinology* **55,** *543 (1954)*). The protein-free diet was prepared with twice the vitamin supplement used in the basal diets of the previous experiment. The plan of the experiments was the same except that some of the rats were autopsied after thirteen days and the remainder after twenty-one to twenty-three days of pregnancy. The condition of the fetuses was observed and the width of the implantation sites was measured.

The daily injection of 0.5 micrograms of estrone beginning on the third day after breeding increased the number of females with living fetuses from about 10 per cent to 22 and 30 per cent when examined at the thirteenth and twenty-first days of pregnancy respectively. A series of experiments was carried out with graded doses of estrone. The maximal maintenance of pregnancies was obtained with 2 micrograms of estrone daily. This dose maintained viable fetuses in 80 per cent of the pregnancies. A beneficial effect could also be demonstrated with the administration of 4 mg. of progesterone daily. With this dose 80 per cent of the females were found to contain viable fetuses, but the average number of live fetuses per rat was only 6, in comparison to a normal of between 8 and 9.

Combined administration of estrone and progesterone maintained live fetuses in 100 per cent of the animals and the number of living young per rat was nearly normal. The improved success of pregnancy in the rats receiving hormone treatment could not be attributed to increased food intake because these animals actually consumed less than did uninjected controls.

The authors interpret their results as indicating that the poor reproductive function of rats on protein-free diets is largely due to the failure of hormone production. The possibility that protein deficiency has increased the hormonal requirement for successful pregnancy is not discussed. The authors speculate concerning the organ responsible for hormonal failure. The defect might be attributable to the failure of gonadotropin production by the pituitary, steroids by the ovary, or gonadotropins by the placenta. The similarity of the effects of protein deficiency and vitamin B_6 deficiency induced by feeding desoxypyridoxine on the course of pregnancy is striking. M. M. Nelson, W. R. Lyons, and H. M. Evans (*Endocrinology* **52,** *585 (1953)*) have found that the administration of estrone and progesterone maintains pregnancy in vitamin B_6 deficiency.

The view that the harmful effect of certain dietary deficiencies on pregnancy is due to hormonal deficiencies rather than to a generalized disturbance of metabolism is important and merits further investigation. The beneficial effects of hormone treatment in protein-deficient rats, reviewed above, are consistent with this theory. The possible clinical application of this information remains to be explored.

THE *IN VITRO* DEPOSITION OF FAT

The metabolism of adipose tissue is of great interest to nutritionists because this tissue acts as a storehouse for dietary calories when intake exceeds requirement. During

periods of caloric insufficiency most of the energy needs of the body are met by mobilization of depot fat. Recent biochemical research has established the metabolic versatility of fat cells, and has displaced the widely held concept of fat cells acting as a passive "coal bin" for fat. The demonstration of the deposition of glycogen in fatty tissues under appropriate experimental conditions by E. Tuerkischer and E. Wertheimer (*J. Physiol.* **100,** *385 (1942)*) suggests that carbohydrate may be taken up by the fat cells and that this carbohydrate may be utilized for fat synthesis within the fat cell.

Direct evidence that fatty acids are removed from the blood and deposited within fat cells has been obtained by incubating fatty tissues with serum. In such experiments, B. Shapiro, D. Weissmann, V. Bentor, and E. Wertheimer (*Metabolism* **1,** *396 (1952)*) have shown that fatty acids are removed from the serum when the flasks are incubated at 38° but not at 20° C. They also found that heating above 60° C. or adding fluoride blocked the uptake of fatty acids. A certain degree of specialization of the function of fat cells is indicated by the observation that subcutaneous connective tissue and diaphragm did not take up significant amounts of fatty acids under identical conditions.

Experiments have been carried out by I. Stern and B. Shapiro (*Metabolism* **3,** *539 (1954)*) to determine the chemical form in which fatty acids are transported in the blood to the adipose cell. In their studies the avidity of mesenteric fat cells for fat was increased by starving rats for five days. The animals were then killed and the incorporation of various lipids by this adipose tissue was studied *in vitro*. When mesenteric fat was incubated with either rat or human serum it was observed that a significant decrease in the total concentration of esterified fatty acids occurred in the medium, but there was no change in the level of phospholipid and cholesterol esters. From these results it was concluded that the fatty acids deposited in the adipose tissue arose from the neutral fat moiety of the serum.

The absorption of individual fatty acid esters was next studied by incubating the fat-depleted adipose tissue in mediums containing 5 per cent albumin and the fatty acid ester to be tested. Under these conditions a significant uptake of triolein was observed but no uptake of lecithin occurred. The absorption of the water-soluble fatty acid esters, sorbitan monolaurate, sorbitan monopalmitate and sorbitan mono-oleate, was faster than the natural neutral ester, triolein. The addition of 0.05 molar sodium fluoride to the incubation flask produced a significant inhibition of the uptake of the esterified fatty acids but did not inhibit the uptake of sodium oleate.

In another set of experiments measurements were made both of the disappearance of sorbitan monopalmitate from the medium and the increase in tissue fatty acids measured after saponification. In most cases the fatty acids which left the medium could be accounted for in the tissue. However the stoichiometry was not very exact. There was no evidence that adipose tissue liberated a lipase into the medium which produced hydrolysis of the ester before absorption into the tissues. When the tissues were stained with Sudan at the conclusion of the incubation, an increase in stainable fat was evident.

Additional evidence that the fatty acids disappearing from the medium were incorporated into the tissue was obtained by incubating adipose tissue with sodium stearate labeled in the first carbon atom with C^{14}. It was observed that most of the isotope which disappeared from the medium could be recovered in alcohol-ether extracts of the tissue. The addition of fluoride to the medium did not inhibit the incorporation. When the radioactivity in the tissue was fractionated it appeared that 30 per cent of the absorbed fatty acid was in ester form after four hours of incubation.

These experiments therefore present evi-

dence that adipose tissue primarily takes up fatty acids from the neutral fat components of the serum. There is no evidence that cholesterol or phospholipids are vehicles for fatty acid transport. The fact that the uptake of fatty esters is inhibited by fluoride, but that the uptake of free fatty acids is not, is interpreted by the authors as indicating that an enzymatic transport mechanism is required for the deposition of fatty acid esters. Because there was no evidence that fatty acid esters were split in the medium it is proposed that the splitting might occur at the cell surface.

The experiments of Stern and Shapiro add evidence of the metabolic complexity of fat cells. The apparent simplicity of the cytoplasm of these cells when seen under the microscope is no measure of their biochemical function.

CHOLINE AND REPRODUCTION

Although a dietary deficiency of choline has been associated with a variety of disturbances, relatively little attention has been paid to its role in the reproductive process. There have been a number of suggestions that choline may be required for normal reproduction, but the observations are not very conclusive. Some of the early work in this field was done by J. W. Hamilton and A. G. Hogan (*see Nutrition Reviews* **2,** *361 (1944)*). They found that when choline was omitted from the breeding diet of the hamster, small but apparently normal litters were born. Most of the young died within one week.

The requirement of the pig for choline during the reproductive process was studied by M. E. Ensminger, J. P. Bowland, and T. J. Cunha (*J. Animal Sci.* **6,** *409 (1947)*) who fed adult female pigs a purified ration containing most of the essential vitamins except choline. They reported that if the choline-deficient diet were started fifty-two days prior to parturition, litters of normal size were born on the scheduled farrowing date. However, the birth weights of the pigs were below normal. The pigs in some litters showed signs of muscular or nervous incoordination. There was a high mortality of the young pigs compared to the group receiving the choline-supplemented ration. It is unfortunate that the alfalfa and grain ration fed the pigs prior to the initiation of the purified diet appeared to be inadequate for reproduction. The pigs were mated while they were on the latter diet. Four of the 18 pigs never conceived—3 of these were in the group that received the vitamin-supplemented purified diet.

M. Peet and M. M. Sampson (*Science* **107,** *548 (1948)*) reported that the uteri of 2 adult female rats that had been maintained on a choline-deficient diet for thirty-nine days showed a complete lack of contractility and a partial atrophy of both the uteri and ovaries. The observations of Peet and Sampson could not be confirmed by C. P. Kraatz and C. M. Gruber (*Ibid.* **109,** *310 (1949)*). The latter workers found no difference in the force of rhythmicity of uterine contractions when the excised uteri were placed in aerated Locke's solution.

L. Mirone (*Am. J. Physiol.* **179,** *49 (1954)*) studied the influence of choline on the reproductive capacity of mice. She used a basal diet containing 23 per cent vitamin-free casein and 15 per cent lard in addition to adequate amounts of salts and all the vitamins except choline. Although she included a variety of dietary modifications, the only diets pertinent to the present discussion are the stock, the purified containing vitamin B_{12} (her diet M 5) and the latter minus choline (her diet M 15). Groups of 20 weanling female mice on the stock and choline-supplemented purified diet gained weight at the rate of 1.5 g. per week, whereas the mice on the choline-deficient ration gained

1.1 g. Since only average weight gains were presented, it is not possible to calculate the significance of these differences. Blood samples taken when the mice were 3 and 7 months of age showed no disturbances so far as hemoglobin, red cells, leukocytes, polynuclear and mononuclear cells were concerned.

After the mice had been on the purified diets for four months, they were bred to fertile males (dietary history not given). Whereas 100 per cent of the mice on the stock diet and 80 per cent on the choline-supplemented diet became pregnant, only 66 per cent on the choline-deficient diet conceived. The average birth weights of the mice born to the mothers on the choline-deficient diet were slightly smaller than those on the stock or choline-supplemented diet (1.2 g. versus 1.4 and 1.3 g. respectively). None of the mice born to the mothers on the choline-deficient diet was alive on the fourth day, whereas half of those on the choline-supplemented and all on the stock diet were living at that time.

By the time they were bred, more than half of the adult female mice on the choline-deficient diet had died, while none of the animals on the choline-supplemented or stock diet succumbed during the seven months of the experiment.

Relatively little work has been done with the mouse insofar as choline is concerned. W. H. Griffith and J. F. Nyc ("The Vitamins," *Vol. II, p. 103*, edited by W. H. Sebrell and R. S. Harris, *Academic Press Inc., New York (1954)*) refer to only three papers on the subject. One of these (D. Stetten and G. F. Grail, *J. Biol. Chem.* **148,** *509 (1943)*) involved a study of the disappearance of deuterium-labeled fats from the livers of mice. A choline-deficient diet was fed one group of animals for fourteen days, resulting in a slight increase in liver fat. Another paper (H. M. Barrett, C. H. Best, and J. H. Ridout, *J. Physiol.* **93,** *367 (1938)*) presented the results of feeding a diet poor in "lipotropic agents" to mice but did not include a group receiving the same diet plus choline. The third is an abstract by E. A. White and L. R. Cerecedo (*Proc. Am. Chem. Soc.* **23B** *(1946)*) reporting that renal lesions could be produced in mice only if the choline-deficient diet were consumed prior to weaning.

The work of Mirone (*loc. cit.*) is important because it indicates that the mouse may require a dietary source of choline even in the presence of a high protein intake. Should this work be confirmed, it would be desirable to study the influence of methionine and other methyl donors as possible replacements for choline in the mouse.

PROTEIN DIGESTIBILITY

The origin and nature of the fecal nitrogen under various conditions has received a good deal of study. Standard textbooks of biochemistry tell us that "food residues of the feces represent a very small proportion of the fecal mass, since under normal circumstances the protein, lipid, and carbohydrate of the diet are rather completely digested and the digestion products absorbed. As much as one-quarter of the dried feces may be represented by bacteria" ("Principles of Biochemistry," A. White, P. Handler, E. L. Smith, and D. Stetten, *p. 514, McGraw-Hill Book Co., New York (1954)*). On the other hand, measures of the fecal excretion of amino acids have been used as a measure of the extent to which amino acids are absorbed (*Nutrition Reviews* **7,** *99 (1949)*; B. S. Schweigert and B. T. Guthneck, *J. Nutrition* **54,** *333 (1954)*). While these studies do show that the fecal amino acid composition may be varied by the kind of protein fed, they do not necessarily show that the fecal amino acids are of dietary origin.

The digestibility of proteins is commonly

measured and usually used as though it were characteristic of the protein itself. It is generally agreed that the fecal nitrogen may be divided into two parts, the metabolic nitrogen being that which occurs upon a nitrogen-free diet, and the undigested nitrogen being the difference between the metabolic nitrogen and the total fecal nitrogen. The latter figure would presumably be related to the protein fed if the method of estimating the metabolic nitrogen is adequate. The extensive studies of B. H. Schneider (*Biochem. J.* **28,** *360 (1934)*), working in Mitchell's laboratory, have shown that the metabolic nitrogen is related to the amount of food eaten.

Evidence accumulating demonstrates that even with the refinements which presumably account for metabolic nitrogen, the figures obtained as protein digestibility may not be valid. By comparing the fecal nitrogen of human subjects receiving diets composed largely of different breads with varying protein and fiber contents, R. A. McCance and E. M. Widdowson (*J. Hyg.* **45,** *59 (1947)*) concluded that the amount of fecal nitrogen was not characteristic of the protein but more likely related to the fiber content. D. M. Hegsted, M. F. Trulson, and F. J. Stare (*Physiol. Rev.* **34,** *221 (1954)*) have summarized some of the other evidence indicating a major role of the fiber content of the diet upon protein digestibility.

E. W. Crampton and B. E. Rutherford (*J. Nutrition* **54,** *445 (1954)*) have contributed further to the problem in a study with rats in which egg or cheese protein was fed at several levels mixed with various amounts of shredded wheat and methylcellulose. These studies showed that most of the variability in the fecal nitrogen was directly traceable to the amount of protein consumed. There was a steady rise in fecal nitrogen as the level of intake increased, but since the fecal nitrogen did not reach zero at zero levels of intake, the percentage of protein absorbed increased with intake. The authors calculated that the available calories per gram of protein were only 2.3 on a 5 per cent protein diet and increased to 4.0 when the diet contained 45 per cent protein.

The authors conclude that differences in apparent digestibility of protein of different kinds are not primarily due to inherent differences in true digestibility, but rather that the fecal nitrogen is essentially all of "metabolic" origin and increases only slightly as more food nitrogen is available for bacterial growth. Thus the level of protein becomes primarily important and "any relation between type of food and the apparent digestibility of its protein is coincidental."

The evidence thus accumulates that digestibility as a characteristic of individual proteins loses its value and the diet with which it is fed becomes the more important factor. Many authors will find it difficult to reconcile the views and experiments of Crampton and Rutherford with the considerable body of data which indicates that the bulk or fiber content of the diet may often be a determining factor in protein digestibility.

SOME EFFECTS OF WATER DEPRIVATION

The effects of water restriction on food intake and food utilization for growth have been reported by E. W. Crampton and L. E. Lloyd (*J. Nutrition* **54,** *221 (1954)*). The study was done on young growing rats, one group receiving water ad libitum, the other group being restricted to one-half the intake of the ad libitum group. Voluntary food consumption was measured over a two-week period. The animals receiving the limited supply of water restricted their food intake by 27 per cent (ranging from 20 to 36 per cent) in four different experiments. Weight gain under these conditions of

inanition was reduced 51 per cent below that of the ad libitum group. Efficiency of food utilization as measured by weight gain per gram of food consumed was reduced by 31 per cent in the water-restricted group. These data express in a quantitative way the casual observations of those who have had extensive experience with animal colony maintenance and animal nutrition experiments.

Quite a different effect of water restriction has been reported by M. Schreiber and C. A. Elvehjem (*J. Nutrition* **54,** *257 (1954)*). In an earlier paper R. A. Collins, Schreiber, and Elvehjem (*Ibid.* **49,** *589 (1953)*; *Nutrition Reviews* **11,** *311 (1953)*) described their observation of red-stained fur of animals deficient in riboflavin, pantothenic acid, or pyridoxine when maintained at a relative humidity of 90 per cent. Schreiber and Elvehjem concerned themselves with the possible effect of certain flavonoids on the secretion of the red pigment caused by such nutritional stresses as vitamin deficiency or water restriction at high relative humidity. The flavonoids such as rutin were of particular interest because of their possible effects on capillary fragility and permeability.

Weanling male rats were fed a synthetic diet either complete or lacking one of several B-vitamins. The animals were observed during a three-week experimental period. The degree of pigment formation was judged on an arbitrary scale. In the first experiment it was found that the inclusion in the diet of three flavonoids (rutin, quercetin and quercitrin) could markedly reduce the production of the red pigment caused by riboflavin or pyridoxine deficiencies and high relative humidity. It was noted that animals receiving the deficient diet reduced not only their food but also their water intakes. This led to a further study of the possible role of food and water restriction in the secretion of the red pigment. Restriction of water intake to 4.0 ml. per day caused some increase in pigment output. Food restriction had no effect. Reduction of both food and water intake did not alter the secretion of pigment. Thiamine and choline deficiencies caused an increase in staining. Niacin deficiency caused an increase of dubious significance.

Since these flavonoids are quite insoluble in water, the possibility existed that they might exert their protective action by virtue of their persistence in the alimentary tract where they might modify the bacterial population. To explore this possibility the flavonoids were injected intraperitoneally. By this route they offered partial protection against the pigment-inducing effect of water restriction or riboflavin deficiency. Penicillin and streptomycin had no protective effect, making somewhat less likely the possibility that the intestinal flora might be responsible.

The authors have carried out a large number of experiments, each with a relatively small number of animals. They have made observations on the complex interplay of environmental and nutritional stresses and on the protective action of certain flavonoids which have held an ambiguous position in the science of nutrition.

NOTES

Letter to the Editor

Dear Sir:

I have noted the reference to our paper "Nutrition and Haemoglobin Surveys in Children in Nilgiris District" (*Indian J. Med. Res.* **42,** *55 (1954)*) on pp. 332–33 in the November 1954 issue of your esteemed journal. The reviewer has offered some criticism which I consider to be not strictly fair. I believe that this is probably due to the fact that the reviewer has not appreciated the object of our investigation.

The main object of our investigation was to see how far the routine assessment schedule recommended by the Nutrition Advisory Committee of the Indian Council of Medical Research could be fruitfully applied for assessing the nutritional status of an individual, and to suggest modifications in the light of our present work, so that the schedule might become more useful in nutrition surveys for regional comparisons and longitudinal studies.

Our recommendations on the suitability of the schedule based on our experience of examining nearly 2300 children are contained in the paper in question. I need not refer to them here.

We were not concerned with somatometric data alone. Clinical signs, no doubt, serve as a good index of the nutritional status, but by themselves they are not enough, since malnutrition can exist without specific signs.

No claim is made in our paper that the somatometric data provide the needed growth norms. Further, it has been admitted at the outset that for lack of standards for Indian children our anthropometric data cannot be compared to evaluate the degree of malnutrition.

However, our experience shows that the growth pattern of the children included in this study follows closely that of a larger group consisting of about 40,000 unselected children from various parts of the country; in fact, at several points the two curves overlap. Further, the trend of growth of Indian children runs parallel to those of their counterparts in the United Kingdom and United States of America, but *at a lower level* as indicated by V. N. Patwardhan (*Calcutta Med. J.* **51,** *117 (1954)*).

We have been attempting to work out growth standards for normal healthy Indian children, but it will be some time before one can speak of growth norms.

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Editorial Comment: We have examined only *one* aspect of the study. This is why our comment could readily appear unfair to the authors while, in fact, it would not be so regarded by readers who saw both the original and our review. We did not say that they claimed to have provided valid growth norms. We simply said that their data, without further evidence, could not be regarded as such.

There is a good deal of difference between these two "claims." Our comment was to serve simply as a warning and a stimulus.

Fluoridation of Salt

Dr. H. J. Wespi has made the interesting suggestion in the *Schweizerische Medizinische Wochenschrift* **84,** *885 (1954)* that salt manufacturers in Switzerland should furnish for the inhabitants of areas where fluoridation of water supplies is not practicable a table salt containing 200 mg. of sodium fluoride and 10 mg. of potassium iodide per kilogram to prevent fluorine and iodine deficiencies and thus lower the incidence of tooth decay and goiter, respectively. The author favors fluoridation of drinking water where this practice is well adapted.

Food Yeast from Potato Waste

Production of yeast as a food supplement has been investigated by C. O. Reiser (*J. Agr. Food Chem.* **2,** *70 (1954)*) from the economic and engineering viewpoint. Wastes from numerous agricultural products have been considered as sources of energy for yeast; they appear to be marginal sources for production of stock feed because of extreme dilution of nutrient material or the necessity to add too much nitrogen, phosphorus, or potassium to permit utilization of the sugars. Reiser has emphasized the use of potato starch wastes, consisting of protein wash water and potato pulp. The 1.7 per cent solids content consists of 38 per cent protein, too dilute for economical recovery; however, the presence of adequate nitrogen and inorganic nutrients suggests the desirability of extraction through yeast cultivation.

It appears that a yeast-producing plant with a 4.5 ton daily capacity could produce yeast at a cost of 5 cents per pound. This would be feasible from the wastes of a 30-ton starch plant. Further study of optimum conditions might reduce the cost indicated. In view of the numerous valuable nutrients (vitamins and proteins) found in yeast, this cost appears attractive. The yeast *Torulopsis utilis* resists contamination by other organisms when the pH is held below 5 and it produces a reasonably palatable product.

Food Acceptance

The Quartermaster Food and Container Institute for the Armed Forces, 1819 West Pershing Road, Chicago 9, Illinois, has issued a report of the proceedings of a symposium entitled "Food Acceptance Testing Methodology." This publication of the National Research Council Advisory Board on Quartermaster Research and Development, Committee on Foods, is being distributed by the Food and Container Institute without charge.

Synthesis of Squalene from Acetate in Man

It has been demonstrated in man and experimental animals that cholesterol can be synthesized in the liver from acetate (*Nutrition Reviews* **12,** *230 (1954)*). The aliphatic hydrocarbon squalene appears to be an intermediate in cholesterol biosynthesis. However, squalene has not been found in mammalian liver. M. L. Eidinoff *et al.* (*J. Clin. Invest.* **33,** *333 (1954)*), noting that small quantities of squalene are present in human sebum, fed C^{14}-labeled sodium acetate to human subjects and examined the sebum for radioactive squalene. The sebum was collected by washing parts of the skin with acetone. The hydrocarbon was extracted from the washings and analyzed. Radioactivity was found in the squalene from sebum and it was demonstrated that the methyl carbon of the acetate was the precursor.

The results of these studies do not prove that squalene is an intermediate in cholesterol synthesis in man but render this a distinct possibility.

Recent Nutrition Books

Report of the Third Conference on Nutrition Problems in Latin America. Sponsored jointly by the Food and Agriculture Organization and the World Health Organization, Caracas, Venezuela, October 19–28, 1953. Food and Agriculture Organization, Rome, Italy, June 1954.

Energy Metabolism and Nutrition. Raymond W. Swift and Cyrus E. French. The Scarecrow Press, Washington, D. C., 1954. Pp. 264.

Bread. The Chemistry and Nutrition of Flour and Bread with an Introduction to Their History and Technology. Lord Horder, Sir Charles Dodds, and T. Moran. Constable and Company, Ltd., 10–12 Orange Street, W.C. 2, London, England. Pp. 181. Price: 18 shillings net.

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